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Carcinogenic risks and chemical composition of particulate matter recovered by two methods: wet and dry extraction

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Abstract Wet and dry extraction methods are two main methods used in toxicological in vitro and in vivo studies to recover particulate matter (PM) from filter papers. The aim of this study was to extract PM by wet and dry extraction methods and compare the elemental content and carcinogenic risks of extracts. PM₁₀ samples were collected using fiberglass filters and a high-volume air sampler. For wet extraction, the method involved agitation in water, sonication in water bath, and agitation again. For dry extraction, the filters were sonicated and the PM was recovered using sweeping by a brush. Elemental composition of extracts was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). Excess lifetime cancer risks (ELCR) of As, Cd, Cr, Ni, and Pb in extracts were estimated. The average recovery efficiency (%) of dry and wet

extraction methods were 36.8% and 58.5%, respectively. The average elemental concentration that resulted from dry and wet methods was calculated to be 2.27 and 1.26 $\mu\text{g}/\text{m}^3$, respectively. The total ELCR of all heavy metals in both methods exceeds the 1×10^{-6} limit. However, the total ELCR of heavy metals that resulted from the dry method was higher than that from the wet method. In conclusion, the dry method showed to be more effective to recover a representative extract from the filter. This can ultimately lead to a realistic and robust response in toxicological studies. However, a toxicological comparison between the extracts of these two methods is required.

Keywords PM₁₀ · Health risk assessment · PM recovery · PM extraction method · Toxicology

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Introduction

Particulate air pollution is associated with many adverse health effects in humans. Several epidemiological studies have indicated that particulate matter (PM) induce cardiovascular diseases and respiratory diseases, etc. (Pope III et al. 2002; Turner et al. 2011; Nabizadeh et al. 2019). Investigating the toxicity and chemical composition of particles requires sampling and extraction of the particulate matter (Faraji et al. 2018; Roper et al. 2015). The PM is sampled by passing the air through a filter to separate the solids from the gas. The recovery or extraction of PM from filter depends on the

research purpose and can be complete or selective (Bein and Wexler 2015).

Toxicological studies mainly tend to have complete extraction of PM and maintain the physical and chemical characteristics of PM for the surveys (Bein and Wexler 2015). PM extraction methods may change the physical state and contribution of chemical species of the extracts compared to the initial sampled particles. This can interrupt the toxicological matrix effect since it alters the composition of particle mixture (Landlová et al. 2014). The results of studies using different extraction techniques should be compared with caution. In addition, recovery efficiency, i.e., the percentage of recovered mass to the sampled mass, can be a critical factor. Higher recovery efficiencies eliminate the need for more sampling or increase the potential for applying higher dosages (Roper et al. 2015).

Several methods have been developed for complete extraction of PM. These methods are using ultrasonic energy (Ashley et al. 2001); aqueous solutions and sonication (Biran et al. 1996); sonication and lyophilization (Devlin 2009; Bowser 2009); water, sonication bath, and agitation (Shi et al. 2003); sonication and sweeping with a brush (Alfaro-Moreno et al. 2002); and a high-efficiency method called multi-solvent extraction (MSE) that incorporates sonication, liquid-liquid extraction, selective filtration, and solvent removal (Bein and Wexler 2015). Application of these methods depends on the purpose of the study and available facilities and instruments (Roper et al. 2015).

Extraction with water, sonication bath, and agitation (wet extraction) (Shi et al. 2003) and sonication and sweeping with a brush (dry extraction) (Alfaro-Moreno et al. 2002) are two common and applicable methods used for PM extraction in previous studies. The chemical composition of extracts from these methods can be different, and eventually alter the toxicological matrix effect of PM. The aim of this study was to extract PM by wet and dry extraction methods and compare the elemental content and carcinogenic risks of extracts.

Materials and methods

Sampling

PM with aerodynamic diameter of 10 μm and less (PM_{10}) was sampled from the top of a building located at Shariati Hospital (35° 43' 16.8" N and 51° 23' 12.3"

E) of Tehran, Iran. The building is near a highway (600 m distance) and two streets with heavy traffic (165 and 100 m distance). The building's height was about 10 m, and no trees or other obstacles were around. The sampling was conducted during the summer 2017 in once every 6 days.

All the stages of study are shown in Fig. 1. Five 24-h PM_{10} samples were collected using the fiberglass filters (20.3 cm \times 25.4 cm) and a high-volume air sampler (Thermo Andersen, USA) operated at a flow rate of 1 m^3/min . In order to remove any pre-contamination, the clean filters were washed with double-distilled water and placed in an oven at a temperature of 50 $^{\circ}\text{C}$ for 10 h before sampling. Initial and final (after sampling) weights of the filters were measured by an analytical balance (Model: Sartorius 2004 MP). Before weighting, the filters and extracts (in the next sub-sections) were maintained under the controlled temperature (20–25 $^{\circ}\text{C}$) and relative humidity (25–30%) for 24 h. All chemical were purchased from Merck and Sigma–Aldrich.

Wet extraction

The procedure for the wet extraction of PM_{10} is presented elsewhere (Shi et al. 2003). Briefly, one-half of the filter was divided into two parts. Each part was extracted separately for elemental and ionic analysis. Each part was cut into 2 \times 2 cm pieces, placed into 10 mL of

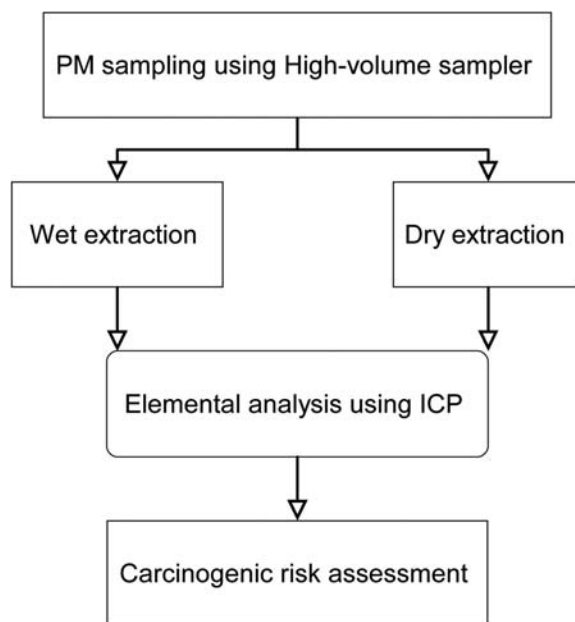


Fig. 1 Flow diagram of the study stages

double-distilled water, agitated for 5 min, sonicated in a water bath for 5 min, and agitated again for another 5 min. The filters were washed with another 10 mL of double-distilled water and the water was added to the extracts. The extracts were dry-vacuumed and weighed.

Dry extraction

The procedure for dry extraction of PM_{10} are presented elsewhere (Alfaro-Moreno et al. 2002; Faraji et al. 2018). Briefly, one-half of each filter was divided into two parts. Each part was extracted separately for elemental and ionic analysis. The filters were dry sonicated for 45 min (Elma-ultrasonic, Germany) and swept by smooth brushing. The dry PM was collected and weighed.

Elemental analysis

In order to determine the concentrations of elemental components, 4 ml HNO_3 (69%), 2 ml $HClO_4$ (70%), and 0.2 ml HF (48%) were added to the dry extracts and digested in a Teflon digestion vessel under high pressure at 170 °C for 4 h. Then, the acidic solution was cooled, dried (90–100 °C), weighed, and diluted with 10 mL of double-distilled water and filtered (0.45 μm pore size). Finally, the filtrate was analyzed by an inductively coupled plasma-optical emission spectrometry (ICP-OES, model: ARCOS, SPECTRO) (Shahsavani et al. 2012; Hassanvand et al. 2015; Sun et al. 2004).

Two laboratory blanks and one field blanks were analyzed. Limit of detection (LOD) was defined as three times the standard deviation of the blank samples. The values of LOD and limit of quantification (LOQ) are presented in Table A1 of the Supplementary material.

Health risk assessment

Excess lifetime cancer risks (ELCR) of some PM-bound heavy metals were estimated using an approach that is fully described in previous studies (Kermani et al. 2018; Park et al. 2008). The data on inhalation unit risk (IUR) of heavy metals were obtained from EPA documents (US EPA 1998). The IUR values used for As, Cd, Cr, Ni, and Pb were 4.3×10^{-3} , 1.8×10^{-3} , 1.2×10^{-2} , 2.4×10^{-4} , and 1.2×10^{-5} ($\mu g/m^3$)⁻¹, respectively. We assumed that all of the heavy metal concentrations are absorbed in the body. Since only Cr(VI) induces cancer risks, and the ratio of Cr(VI) to Cr(III) in the air is about

1 to 6, the total concentration of Cr measured by ICP was divided by 7 to obtain only the concentration of Cr(VI) for risk assessment analyses. The following equation was used to calculate the ELCR:

$$\text{ELCR} = \text{heavy metal average concentration} \times \text{inhalation unit risk} \quad (1)$$

Statistical analysis

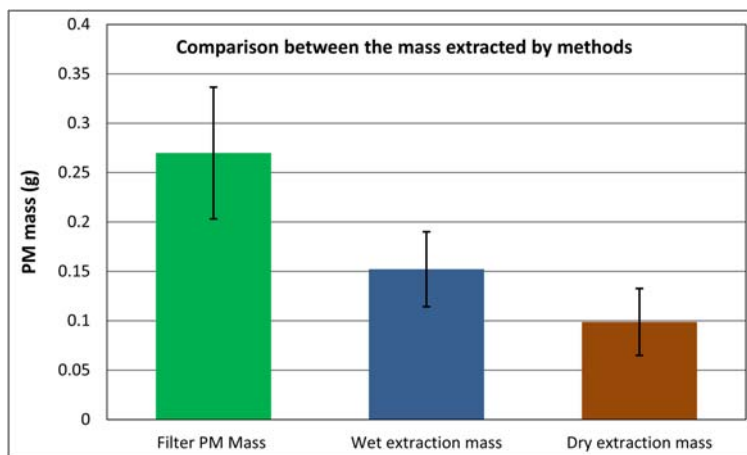
To compare the mass of elemental and ionic mass recovered by two methods, concentrations of all elements or ions from each filter were summed. First, the normality and homogeneity of variances were analyzed using Kolmogorov-Smirnov and Levene's test, respectively. Then independent *t* test or Mann-Whitney test was used for parametric or non-parametric conditions, respectively. R Programming software was used for statistical analysis.

Results

Two different methods for PM extraction from sampling filters were used and had undergone elemental and ionic analysis. Figure 2 shows the comparison between the mass extracted by dry and wet methods with total PM mass in filters. The average recovery efficiency (%) of dry and wet extraction methods were 36.8% and 58.5%, respectively. The elemental composition of extracts from two methods is presented in Table 1. In case of most elements, the mass recovered by the dry method was more than that recovered by the wet method. In total, the average elemental mass that resulted from dry and wet methods were calculated to be 2.27 and 1.26 $\mu g/m^3$, respectively. Statistical analysis showed that there is a statistically significant difference between the elemental mass recovered by these two methods (*p* value = 0.03).

Figure 3 shows the ELCR values of As, Cd, Cr, Ni, and Pb concentrations recovered from dry and wet extraction method. According to this figure, the total ELCR of all heavy metals in both methods exceeds the 1×10^{-6} limit. However, the total ELCR of heavy metals that resulted from the dry method was higher than that from the wet method. In addition, the cancer risks of Cd and Pb were in levels that can be considered as negligible (below 1×10^{-6}).

Fig. 2 Comparison between the mass extracted by dry and wet methods with total PM mass in filters



Discussion

The PM₁₀ sampled on filters were extracted using the wet and dry extraction methods, and the elemental contents were compared. The wet extraction method showed to have higher recovery efficiency than the dry method. In another study, Ghanbarian et al. (2018)

Table 1 Elemental composition of extracts from dry and wet methods

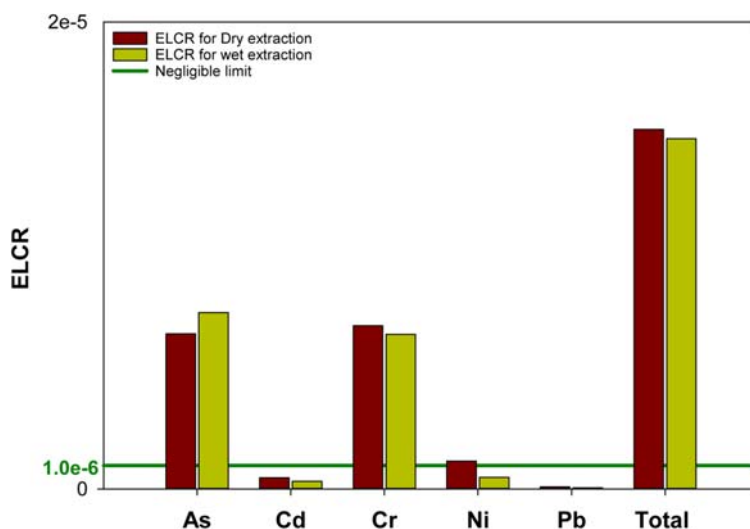
Elements	Unit	Dry extraction	Wet extraction
Ag	ng/m ³	0.31 (± 0.26)	0.67 (± 0.59)
Al	μg/m ³	0.28 (± 0.21)	0.11 (± 0.07)
As	ng/m ³	1.54 (± 2.1)	1.75 (± 2.97)
Ba	μg/m ³	0.67 (± 0.44)	0.38 (± 0.17)
Cd	ng/m ³	0.26 (± 0.23)	0.17 (± 0.13)
Co	ng/m ³	0.43 (± 0.44)	0.23 (± 0.07)
Cr	ng/m ³	4.07 (± 4.78)	3.86 (± 2.97)
Cu	ng/m ³	72.62 (± 63.02)	104.86 (± 72.22)
Fe	μg/m ³	0.46 (± 0.41)	0.22 (± 0.06)
Hg	ng/m ³	0.06 (± 0.06)	0.03 (± 0.02)
Li	ng/m ³	1.21 (± 0.87)	0.24 (± 0.09)
Mn	ng/m ³	15.24 (± 12.5)	10.02 (± 1.71)
Mo	ng/m ³	0.11 (± 0.15)	0.04 (± 0.04)
Ni	ng/m ³	4.96 (± 9)	2.06 (± 1.53)
Pb	ng/m ³	7.61 (± 4.58)	4.19 (± 1.94)
Sn	ng/m ³	1.21 (± 1.12)	0.47 (± 0.19)
V	ng/m ³	1.03 (± 0.86)	0.65 (± 0.14)
Zn	μg/m ³	0.73 (± 0.41)	0.4 (± 0.19)
Total	μg/m ³	2.27 (± 1.47)	1.26 (± 0.46)

Italicized numbers indicate higher values compared to the other extraction method

reported that recovery efficiency of sonication and brushing was in the range of 30–50% (Ghanbarian et al. 2018), which is consistent with our results. In addition, the recovery efficiency of wet extraction method is reported to be 60–70% (Bein and Wexler 2014). This lower recovery percentage in dry method rather than wet method can be due to the procedure of dry method, in which a brush is used to recover the sampled PM (Faraji et al. 2018). Since most of the particles are gone through the filter texture, and/or attached to the fibers, sonication and brushing cannot detach them effectively. For a more efficient extraction, the brush can be swept with more pressure, but this leads to contamination of extracted PM with detached fibers. The presence of fibers in the extracted mass can induce toxicological effects and disrupt the next analyses (Roper et al. 2015).

Dry extraction method showed to extract elemental mass 1.8-fold more than the wet method. Although we showed that the total mass recovered from dry method was lower than that from the wet method, it can recover greater amount of elements. This is mainly because the wet method uses water as the solvent, and elements have low solubility in neutral water (Başak and Alagha 2010). In fact, most of the mass recovered from this method is probably water-soluble ions. On the other hand, dry method recovers PM from the filter, regardless of the chemical composition. This causes to yield an extract representative for the actual particulate matter sampled. This is an important issue in toxicological studies (Roper et al. 2015). The main aim of PM recovery in toxicological studies is to maintain the physical and chemical characteristics of the particles as it is initially presented in the atmosphere (Bowser 2009). It

Fig. 3 ELCR values of some heavy metal concentrations recovered from dry and wet extraction method



seems that dry method is more compatible with this objective rather than the wet extraction method.

In the study, the efficiency of two methods including multi-solvent extraction (MSE) and spin-down extraction (SDE) for recovery of PM was analyzed, and the mass closures for MSE and SDE were obtained at about 92–95% and 36–52%, respectively. MSE includes a combination of sonication and series of solvents to extract multiple compositions of particles (Bein and Wexler 2015). In addition, significant variations were found between the composition (metals, water-soluble ions, polycyclic aromatic hydrocarbons, non-aromatic organics, elemental carbon, and organic carbon) of extracts from two methods (Bein and Wexler 2015). Roper et al. (2015), using an extraction method involving ultrasonic waves and methanol as the solvent, found that there were different recovery efficiencies for metallic and organic species, with many organics lost completely during extraction (Roper et al. 2015). A toxicological comparison of MSE and SDE showed that SDE induced more severe effects in mice rather than MSE (Van Winkle et al. 2015), probably due to the different recovery efficiencies for each compositional fraction of PM and matrix effect (Bein and Wexler 2015). In case of dry and wet extraction methods, there is a need for a toxicological study to compare the effect of extracts.

Although we did not perform a toxicological research, a risk assessment approach was carried out to compare the carcinogenic risks of heavy metal contents extracted by dry and wet methods. Dry method had higher carcinogenic risk than the wet method. It is simply because of higher content

of heavy metal in the dry-extracted PM. In addition, the total carcinogenic risk of heavy metals from both methods exceeded the 1×10^{-6} limit set by US EPA. According to US EPA, the ELCR should be less than 1×10^{-6} (US EPA 1990; 1989; 2005). This shows that the amount of As, Cd, Cr, Ni, and Pb in the extracts of dry and wet extraction methods are in a level that can cause possible carcinogenic risk in human. However, the synergistic and antagonistic effects of the simultaneous presence of various compounds can lead to some variations in toxicological responses (Dong 2018). In a study by Motesadi Zarandi et al. (2018) in Tehran, PM_{2.5}-bound heavy metals were extracted using acidic digestion. Carcinogenic risks for Ni and Pb were below 1×10^{-6} and for Cr and Cd were greater than 1×10^{-6} (Zarandi et al. 2018). These results are mostly consistent with those from our study.

Table 2 presents the summary of advantages of the two extraction methods based on the results of the present study. As it was mentioned before, more PM mass was recovered by wet extraction, but the mass of elements was less than the other method due to lower solubility of

Table 2 Characteristics of the two extraction methods based on the results of present study

Dry extraction	Wet extraction
More recovered elemental mass	More recovered PM mass
Higher estimated carcinogenic risks	
Providing actual chemical composition of PM	
Suitable for toxicological studies	

elements in water. Therefore, dry extraction method recovers the actual chemical composition of PM that is necessary for toxicological studies (Hadei and Naddafi 2020). Maintaining the physical and chemical characteristics of PM is essential for in vitro and in vivo studies (Bein and Wexler 2015). Any change in these characteristics may interfere the toxicological matrix effect (Landlová et al. 2014). In addition to the applications in environmental control, the results of this study are one step to clarify the role of extraction methods on the chemical composition and health risks of recovered PM mass.

Conclusion

In this study, two different methods were used to extract the particulate matter from filter. These methods incorporate different procedures, one uses sonication and sweeping (dry method), and another uses sonication and solubilizing by water (wet extraction). Although the wet method recovered greater amount of PM mass from filter, the elemental content in the extracts of dry method was more than that in the wet method. The carcinogenic risks of heavy metals from both methods exceeded the 1×10^{-6} limit. The dry method showed to be more effective to recover a representative extract from the filter. This can ultimately lead to a realistic and robust response in the toxicological studies. However, a toxicological comparison between the extracts of these two methods is required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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